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The effect of PCBs on glycogen reserves in the eastern oyster *Crassostrea virginica*

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Abstract

Recent declines in Chesapeake Bay oyster populations have been attributed to disease, and reduced water quality from pollution. The stress associated with pollutant exposure may reduce energy available for growth and reproduction. Polychlorinated biphenyls (PCBs) are lipophilic contaminants that may potentially affect mobilization of lipid reserves, increasing reliance on glycogen stores, which could otherwise be utilized to supply energy for gametogenesis. Thus, PCBs may indirectly affect glycogen stores in oysters in a deleterious manner. To test for this effect, reproductively inactive oysters were exposed to PCBs by feeding individuals 0.7 g of algal paste containing 0, 0.35, or 3.5 μg PCBs daily for 8 weeks. Additionally, a group of oysters was exposed to PCBs (0, 0.35, and 3.5 μg) plus 0.3 g of non-toxic artificial sediment to examine interactive effects of sediment particles and PCBs. Adductor muscle, mantle, and gonadal tissues were analyzed for glycogen content. Results suggest that glycogen content is reduced in the adductor muscle with increasing PCB exposure, but there are no effects of PCBs in the mantle and gonadal tissues.

Keywords: Polychlorinated biphenyls; Glycogen reserves; Gonad; Adductor muscle; Mantle; Oysters; *Crassostrea virginica*

Polychlorinated biphenyls (PCBs) pose a serious threat to marine organisms due to their chemical stability and persistence in marine environments. Because PCBs are lipophilic, they accumulate in lipid-rich tissues, and are subsequently transferred up trophic levels (Borlakoglu & Haegele, 1991). PCBs adversely affect lipid metabolism (Ferreira & Vale, 1998; Madureira et al., 1993). With the high-energy yield of

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lipid metabolism potentially reduced, glycogen may be needed to supplement energy metabolism. Glycogen supplies energy for reproduction and anaerobic metabolism. Contaminant exposure may affect glycogen metabolism, resulting in decreased condition index (Pridmore, Roper & Hewitt, 1990). Interaction between pollution and natural stress may increase energy expenditure and anaerobic metabolism (DeZwaan & De Kock, 1988). In mussels, PCB exposure decreased glycogen levels and anoxic survival (Veldhuizen-Tsoerkan, Holwerda & Zandee, 1991). We examined the effects of PCB exposure on glycogen content in the eastern oyster *Crassostrea virginica*. The effect of sediment loading was also simulated to examine its interaction with contaminant exposure. Tissue glycogen content was expected to decrease in PCB-exposed oysters, due to metabolic impairment.

Reproductively inactive oysters were placed in individual 2-l containers containing aerated 1-μm filtered York River water. Oysters were fed daily 0.7 g of algal paste containing 0, 0.35, or 3.5 μg PCBs. PCB-sorbed algal paste was prepared by mixing PCBs (1:1:1 mixture of Aroclor 1242, 1254, and 1260) dissolved in acetone with algal paste (5 mg/g algal paste). To determine the absorption efficiency of PCBs by algal paste, PCBs were extracted from the filtered paste and analyzed for PCBs. The PCBs were found readily sorbed (>99.8%) to the algal paste. Additionally, a second group of oysters was exposed to 0.3 g sediment (Illite, green shale; Wards, Rochester, NY) per day to examine interactive effects between PCBs and sediment. After 8 weeks exposure, oysters ($n = 6$–11/treatment) were sacrificed and tissues (adductor muscle, gonad, and mantle) were excised and frozen in liquid N_2, lyophilized, and weighed. Tissues were homogenized and analyzed for glycogen content using the anthrone reagent method (Baturo, Lagadic & Caquet, 1995). Data were transformed ($\log x + 1$) to meet the assumptions of normality and homogeneity of variance for analysis of variance (ANOVA). Data were analyzed by two-factor ANOVA for effects of PCB dose, sediment and interactions. The Tukey test for multiple comparisons compared means when ANOVA was significant.

PCB exposure significantly affected adductor muscle glycogen ($F_{1,44} = 7.04$, $P = 0.002$). Sediment effects were not significant, but interaction between PCB and sediment was significant ($F_{2,44} = 5.92$, $P = 0.005$). Control (C) and high PCB dose (H) were significantly different from each other (Tukey test, $P = 0.0011$). Significant differences between means are indicated in Fig. 1. In general, muscle glycogen contents decreased as PCB exposure increased. Glycogen in control–sediment (CS) and high-sediment (HS) treatments were higher than the equivalent non-sediment treatments (C, H). This effect was significant in the high PCB dose (HS > H, $P = 0.0453$; see Fig. 1).

Sediment significantly affected mantle glycogen ($F_{1,43} = 5.31$, $P = 0.026$; Fig. 2). PCB and interaction effects were not significant. Mantle glycogen was highest at 3.5 μg PCB + sediment [mean = 89.7 ± 20.11 (S.E.M.) μg glycogen/mg dry wt.]. In the gonad, glycogen contents were not significantly affected by either PCB or PCB + sediment treatments (data not shown).

PCBs accumulate in all oyster tissues as exposure increases (Chu, Soudant, Cruz-Rodriguez & Hale, 1999). PCBs may indirectly affect glycogen metabolism, due to inhibition of lipid metabolism and reproductive processes. The reproductive cycle in

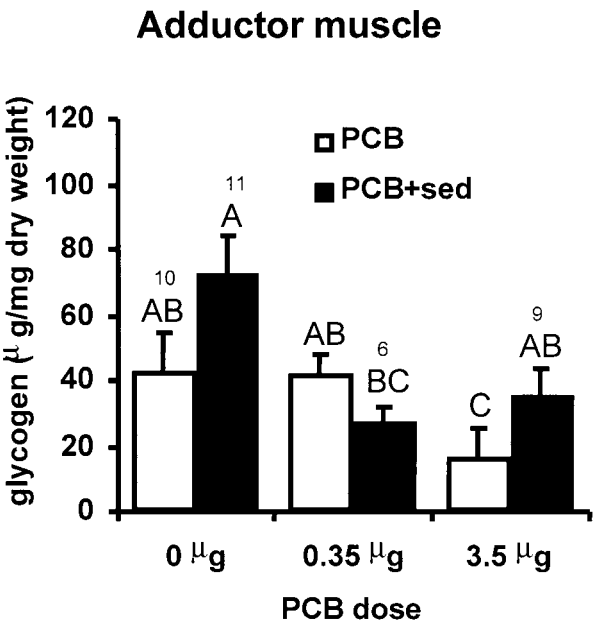

Fig. 1. Adductor muscle glycogen content in oysters exposed to polychlorinated biphenyl (PCB)-sorbed algae at 0, 0.35, and 3.5 μg, and PCB + 0.3 g sediment. Data are presented as mean ± S.E. $n = 7$ unless otherwise noted above bars. Shared letters indicate means that are not significantly different from each other.

oysters involves accumulation of glycogen during periods of high food availability. Glycogen stores are then converted to lipid, which supplies energy for developing gametes. If lipid metabolism is altered during gametogenesis, more glycogen may be required for conversion to lipid. Additionally, alteration of lipid metabolism by PCBs may increase dependence on glycogen to support maintenance metabolism, reducing the amount of glycogen available to support the gametogenesis. Natural stress occurring during either of these two periods could exacerbate PCBs' effects on glycogen reserves.

We expected increasing concentrations of PCBs to decrease glycogen content and condition in all tissues. However, no significant differences between PCB-exposed and non PCB-exposed oysters were observed in condition index (data not shown). Only adductor muscle glycogen decreased significantly with PCB exposure. In the PCB + sediment treatment groups, mean glycogen content decreased from control levels, although the decrease in oysters exposed to 3.5 μg PCB was not statistically significant. We speculate that adductor muscle glycogen may be the initial energy store utilized during stress, while glycogen stores in other tissues (e.g. gonad/visceral mass) may be reserved for reproductive processes, except under extreme or chronic stress. In the bay scallop, *Argopecten irradians*, the adductor muscle is the primary organ of glycogen storage (Barber & Blake, 1991).

Mantle and gonad tissues showed no significant effects of PCBs. The larger goals of this experiment were to condition oysters to gametogenesis and examine the

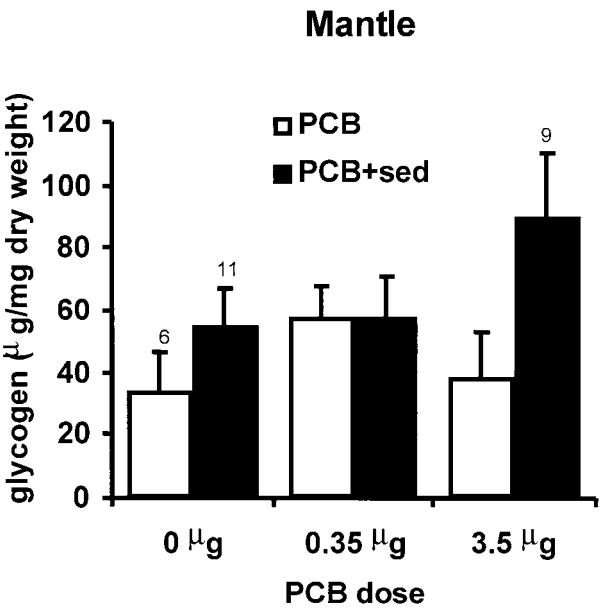

Fig. 2. Mantle glycogen content in oysters exposed to polychlorinated biphenyls (PCBs) and PCB + 0.3 g sediment. Untransformed data are presented as mean ± S.E. $n = 7$, unless otherwise noted above bar. Sediment treatment significantly affected mantle glycogen content ($P = 0.026$).

effects of PCBs on the sex ratio and spawning capability of the oysters. Thus, the feeding regimen was in excess of the oysters' normal requirements to support somatic production. This likely confounded potential effects of PCBs on glycogen reserves, particularly in the developing gonad, as increased feeding and assimilation may have offset deleterious effects of the PCB treatments. Glycogen in gonadal tissues may be devoted solely to reproduction, and not mobilized except under extreme stress. PCBs may have affected reproductive processes without affecting glycogen stored in the gonad. In female sea stars, PCBs lowered gonadal indices but glycogen levels were not affected (den Besten, Herwig, Smaal, Zandee & Voogt, 1990). Gonadal index was not measured in our experiments so direct reproductive effects remain speculative. However, there were fewer females among PCB-exposed oysters.

Sediments were expected to increase particle sorting by the oysters, increasing metabolic demand and decreasing glycogen. Coupled with the additional stress of PCBs, an additive effect was expected. Conversely, sediment treatment increased glycogen content in mantle and adductor muscle in the CS and HS treatments. The cause of this effect is unclear. The additional sediment load may have interfered with the oysters' assimilation of algae, reducing total intake of PCBs, or sorption of PCBs on to sediment may have occurred. PCB analysis in sediment-exposed oysters is currently being conducted. Increases in suspended material have been associated with decreased feeding rates in *C. virginica* (Loosanoff & Tommers, 1948).

Endocrine disruption by PCBs may impair glycogen catabolism. Yellow perch from a PAH and PCB contaminated site exhibited higher liver glycogen contents

than perch from a reference site. This difference was attributed to inhibition of blood cortisol, a hormonal signal necessary to initiate glycogenolysis (Hontela, Dumont, Duclos & Fortin, 1995). Disruption of hormonal function may explain higher glycogen levels in the oyster's mantle tissue (HS treatment), but PCB effects were not significant.

Results show that PCBs significantly affected glycogen in the adductor muscle. High PCB dose decreased glycogen content, possibly indicating the role of this tissue in stress response. Additionally, suspended sediments may play a role in uptake of PCBs, affecting glycogen metabolism.

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